Effect of Ruminally Degraded Protein on Protein Available at the Intestine Assessed Using Blood Amino Acid Concentrations[1,2]

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ABSTRACT: The effect of increasing amounts of ruminally degraded protein on protein available at the intestine was studied using the concentration of blood plasma branched-chain amino acids as an indicator of protein flow to the small intestine. Five ruminally cannulated cows in midlactation were used in a 5 × 5 Latin square design experiment and were fed a diet containing 20% alfalfa silage, 40% corn silage, 30% shelled, coarsely ground corn, and 8% soybean meal (DM basis). Each experimental period was 17 d. Each period contained subperiods: 1 (5 d), 2 (5 d), and 3 (7 d). In addition to the basal diet, cows were given casein infusions of 0, .5, 1.0, 1.5, and 2.0 kg/d. During subperiod 1, the full amount of casein was infused into the abomasum (A100); in subperiod 2, 10% of each casein level was infused into the abomasum (A10); and, during subperiod 3, the full amount of casein was infused into the rumen (R100). The concentration of branched-chain amino acids (Ile, Leu, and Val) in blood plasma increased as amounts of casein infused into the abomasum increased to the full amount. There was no increase in branched-chain amino acids when the same amount of casein was infused into the rumen, suggesting that ruminally degraded protein was adequate in the basal diet and the increased supply of degraded protein from ruminally infused casein did not increase the flow of protein to the small intestine.

Key Words: Cows, Rumen, Protein, Amino Acids, Milk, Carbohydrates

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Introduction

Protein available for absorption in the ruminant intestine is derived from ruminal microbes and dietary protein that escapes degradation during passage through the rumen. Microbial protein can account for 40 to 80% of the total amino acids (**AA**) available for absorption. The major nutrients supporting microbial growth in the rumen are carbohydrate and protein, and protein supplies the essential peptides, AA, or ammonia.

Satter and Slyter (1974) observed that increasing ammonia N concentration beyond 5 mg/100 mL of ruminal fluid did not further increase microbial protein synthesis. Many species of ruminal bacteria are able to use ammonia for growth, but some require peptides and amino acids (Argyle and Baldwin, 1989). Hoover and Stokes (1991) reviewed in vitro experiments that used continuous culture fermenters and reported that total carbohydrate digestion by ruminal microbes was not affected by the proportion of nonstructural carbohydrates (**NSC**). However, efficiency of microbial growth (grams of microbial N per kilogram of carbohydrate digested) increased linearly as the content of ruminally degraded protein (**RDP**) in the diet increased, suggesting that increasing RDP above amounts recommended by NRC (1989) could result in increased efficiency of microbial growth and therefore an increase in the flow of protein to the small intestine.

The objective of this study was to determine the effect of increasing amounts of RDP on concentrations of branched-chain AA (**BCAA**) in blood plasma, an indirect indicator of protein supply to the intestine.

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[2]Trade names and the names of commercial companies are used in this report to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the USDA or an endorsement over products not mentioned.

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Materials and Methods

Five Holstein cows in midlactation and fitted with ruminal cannulas were housed in a stanchion barn in early summer for the duration of the experiment. Local anesthesia was used during surgical installation of the ruminal cannulas. Cows were randomly assigned to treatments in a 5 × 5 Latin square design experiment. Each experimental period was 17 d. Each period contained three subperiods: 1 (5 d), 2 (5 d), and 3 (7 d). The adaptation period was the first 3 d of subperiods 1 and 2 and the first 5 d of subperiod 3. Measurements were taken during the last 2 d of each subperiod.

Cows were fed a diet containing 20% alfalfa silage, 40% corn silage, 30% coarsely ground shelled corn, 8% soybean meal, 1.1% dicalcium phosphate, and .7% trace-mineralized salt (DM basis). A vitamin supplement was added to provide 104,280, 34,760, and 140 IU/d of vitamins A, D, and E, respectively, per cow. Diets were fed as a total mixed ration twice daily. The chemical composition of feed ingredients is reported in Table 1. Orts were restricted to 5 to 10% of intake. In addition to the basal diet, cows were infused with 0, .5, 1.0, 1.5, and 2.0 kg of casein/d into the gut. During subperiod 1 of each period, casein was infused into the abomasum (**A100**). In subperiod 2, 10% of each casein level was infused into the abomasum, assuming that 10% of the total casein infused into the rumen would escape degradation during passage through the rumen (Broderick, 1978). With this assumption, the response in blood plasma BCAA from casein infusion into the rumen (R100) should be approximately equal to the response during casein infusion into the abomasum at a rate equal to 10% of each infusion level (**A10**). This result would occur if casein infusion into the rumen had no effect on microbial protein synthesis. During subperiod 3, the full amount of casein was infused into the rumen (**R100**). A 10% solution of sodium-caseinate was prepared daily and infused continuously into the abomasum and rumen as described by Dhiman et al. (1993), except when cows were in the milking parlor (.5 h/d).

Feed offered and orts for individual cows were weighed daily. Samples of feed ingredients were collected once during each subperiod. Samples of orts were collected daily for individual cows, and a composite sample from each subperiod was used for chemical analysis. The DM concentration in feed ingredients was determined by oven-drying at 60°C for 48 h. Diet formulations were adjusted during each subperiod (if necessary) to account for small changes in ingredient DM concentration. Dried feed samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) using a 1-mm screen and then analyzed for CP by the Kjeldahl procedure, NDF by the modified procedure of Robertson and Van Soest (1980), and ADF according to Goering and Van Soest (1970). During analysis, the samples were further dried at 105°C to determine absolute DM, and chemical analyses were expressed on this final DM.

During the last 2 d of each subperiod, milk samples were collected from four consecutive milkings, and weighted composite samples of a.m. and p.m. milkings were analyzed for fat, protein, and lactose by infrared analysis (Wisconsin DHIA Laboratory, Appleton, WI). Concentration of nonprotein N was determined in 10 mL of milk by deproteination with 10 mL of 30% (wt/vol) trichloroacetic acid (**TCA**); then the sample was mixed, centrifuged at 3,444 × *g* for 15 min at 4°C, and filtered through Whatman number 1 filter paper (Whatman, Clifton, NJ). The protein-free supernatant was stored at −20°C until it was analyzed for N with the micro-Kjeldahl procedure; TCA served as the control. During the last 2 d of each subperiod, blood samples (15 mL) were collected in heparinized vacutainers from the coccygeal vein or artery at 5 h after feeding. Blood samples were centrifuged on the same day at 2,200 × *g* for 15 min at 4°C to separate plasma. Glucose and urea concentrations were determined colorimetrically in deproteinized blood plasma samples (Industrial Method Number 120-71A and 856-87T on a Technicon Traacs 800® Analyzer; Bran + Luebbe Inc., Buffalo Grove, IL). Amino acid concentrations were determined in deproteinized plasma using an amino acid analyzer (Model 6300; Spinco Division, Beckman Instruments, Palo Alto, CA). Lithium was used as a buffer and S-2-aminoethyl cysteine as an internal standard. During the last day

Table 1. Chemical composition of feed ingredients and basal diet

Ingredient	DM	CP	NDF	ADF	RUP[a]	NSC[b]
	% DM basis				% of CP	% of DM
Alfalfa silage	50.5	20.4	38.7	32.5	23	24
Corn silage	35.8	8.5	43.9	23.1	31	34
Dry shelled corn	90.2	9.3	ND[c]	ND[c]	52	74
Soybean meal	90.4	46.9	ND[c]	ND[c]	35	27
Basal diet	60.7	14.0	28.8	17.4	33.9	42.9

[a]From NRC (1989). RUP = ruminally undegraded protein.
[b]Nonstructural carbohydrate (NSC) estimates were from the study of Mertens (1988).
[c]Not determined; NDF and ADF were 9 and 10% for corn and 3 and 10% (percentage DM basis), respectively, for soybean meal.

of each subperiod, strained rumen liquor samples were collected at 5 h after feeding. Rumen pH was measured immediately after collection. Concentrations of ammonia and free AA in ruminal fluid were determined using an alkaline phenol-hypochlorite colorimetric procedure (Broderick and Kang, 1980). Ruminal fluid samples (10 mL) were acidified with formic acid (1:1, vol/vol) and frozen before preparation and analysis for VFA. Analysis of VFA was done using a gas chromatograph (Varian Vista 6000; Varian Instrument Group, Walnut Creek, CA) as described by Brotz and Schaefer (1987).

Data were analyzed using the General Linear Models procedure of SAS (1988). The following model was used for analysis: response = overall mean + cow + period + treatment + (cow × period × treatment interaction) + subperiod + (subperiod × treatment interaction) + error, where response is a dependent variable. The data were analyzed separately for each subperiod when subperiod × treatment interaction was significant ($P < .05$). Least squares means were compared using protected least significant difference.

No evidence was found for nonlinear effects ($P > .05$) of level of casein infusion on ruminal ammonia, plasma urea, milk nonprotein N (**NPN**), and plasma AA concentrations. The slope of the response lines (unit change in response due to a unit change in level of casein infusion) were estimated and compared between subperiods. Significance was declared at $P < .05$ unless otherwise noted.

Results and Discussion

The basal diet contained 14% CP, 9.3% RDP, and 42.9% NSC on a DM basis (Table 1). The calculated TDN content (NRC, 1989) of the diet was 72%, and the NSC:RDP ratio in the basal diet was 4.6 (Table 2). Continuous infusion of five levels of casein into the rumen during subperiod 3 (R100) resulted in NSC: RDP ratios varying between 4.6 and 2.4.

Subperiod × treatment interaction was not significant for nutrient intake, milk yield, milk composition, and glucose concentrations in blood plasma. Therefore, data pooled across subperiods 1, 2, and 3 are presented in Tables 2 and 3. Dry matter intake was decreased with the highest level of casein infusion into the abomasum or rumen ($P < .05$). The decrease in DMI was also reflected in daily NE_L, RDP, ruminally undegraded protein, and NDF intakes. This decrease in intake is probably a reflection of the caloric load of casein infused at the higher infusion rate (Vik-Mo et al., 1974).

Rate of casein infusion had no effect on milk yield, milk composition, or concentration of glucose in blood plasma (Table 3). The cows were in midlactation and probably had a sufficient supply of nutrients from the diet to meet their requirements; therefore, the addi-

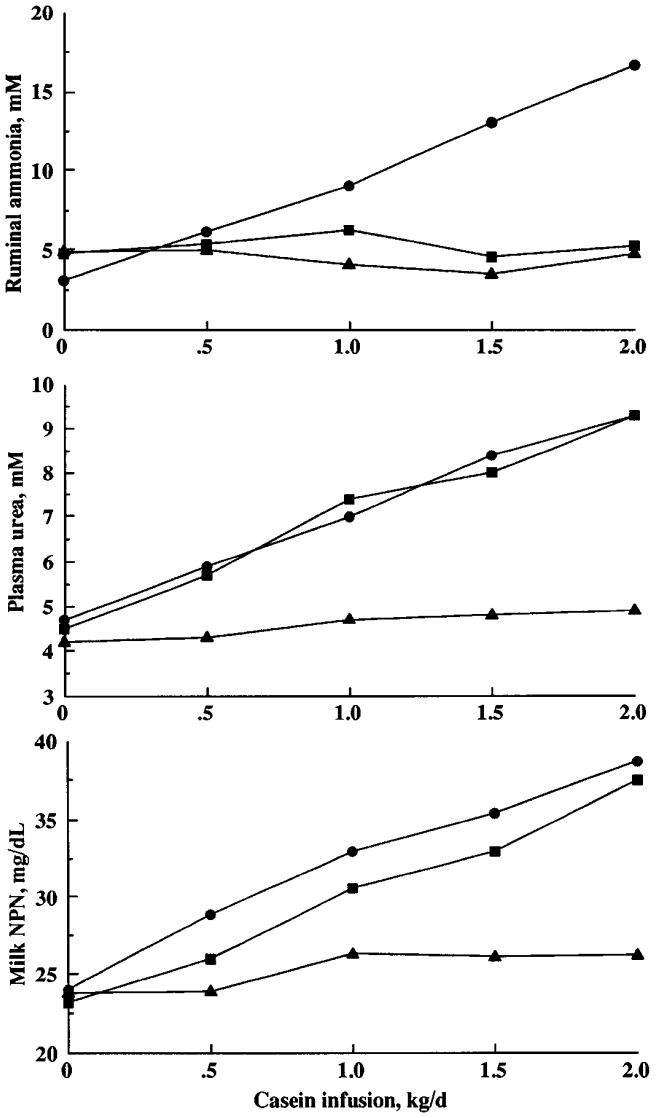

Figure 1. Effect of ruminally degraded protein on concentrations of ammonia in ruminal fluid, urea in blood plasma, and nonprotein N (NPN) in milk. In addition to the basal diet, cows were given casein infusions of 0, .5, 1.0, 1.5, and 2.0 kg/d. During subperiod 1, the full amount of casein was infused into the abomasum (A100, ■); in subperiod 2, 10% of each casein level was infused into the abomasum (A10, ▲); and during subperiod 3, the full amount of casein was infused into the rumen (R100, ●). The SEM for A100, A10, and R100 were 1.4, .7, and 1.0 for ammonia; .2, .2, and .3 for urea; and .8, .6, and 1.0 for NPN in milk, respectively. The slopes of response lines (unit change in response due to a unit change in level of casein infusion) are given below.

	Subperiod			
Item	A100	A10	R100	SE of estimate
Ruminal ammonia	–.017[b]	–.150[b]	3.418[a]	.350
Plasma urea	1.185[a]	.190[b]	1.174[a]	.09
Milk NPN	3.565[a]	.7136[b]	3.582[a]	.278

[a,b]Means with different superscript in the same row differ ($P < .05$).

Table 2. Nutrient intake of cows during casein infusion into the abomasum and rumen[a]

Variables	Casein infused[b], kg/d					SEM
	0	.5	1.0	1.5	2.0	
DM	23.5[g]	22.9[gh]	23.9[g]	22.0[gh]	21.2[h]	.6
NE_L[c]	37.0[g]	36.0[gh]	37.5[g]	34.7[gh]	33.3[h]	1.0
RDP[d]	2.17	2.10	2.21	2.04	1.99	.07
RUP[e]	1.11	1.08	1.14	1.05	1.02	.03
NDF	6.4	6.2	6.6	6.0	5.8	.2
NSC:RDP[f]	4.6	3.9	3.3	2.8	2.4	—

[a]Data were pooled across subperiods because there were no subperiod × treatment interactions ($P < .05$).
[b]In addition to the basal diet, cows were given casein infusions of 0, .5, 1.0, 1.5, and 2.0 kg/d. During subperiod 1, the full amount of casein was infused into the abomasum (A100); in subperiod 2, 10% of each casein level was infused into the abomasum (A10); and during subperiod 3, the full amount of casein was infused into the rumen (R100).
[c]Does not include energy cows received from infusion of casein.
[d]Ruminally degraded protein.
[e]Ruminally undegraded protein.
[d,e]Does not include protein that cows received from casein infusion.
[f]Ratio of nonstructural carbohydrate (NSC) to ruminally degraded protein during casein infusion into the rumen (R100).
[g,h]Means in the same row with different superscripts differ ($P < .05$).

tional casein did not change the yield of milk and milk components. However, administration of casein into the abomasum often increases the yield of milk and milk protein (Clark, 1975), particularly of early-lactation cows. Had the cows in this experiment been deficient in protein, a milk production or milk protein response would have been likely. Ruminal pH, concentration of total VFA, individual VFA, and free AA in the ruminal fluid did not change during casein infusion into the abomasum (A100 or A10). Therefore, only the data for rumen measurements during casein infusion into the rumen (R100) are given in Table 4. Ruminal pH showed no change during casein infusion into the rumen. Casein infusion into the rumen slightly decreased acetate and increased the proportion of isobutyrate, isovalerate, and valerate, indicating the conversion of protein to branched-chain VFA in the rumen (Broderick, 1986). Concentrations of free AA in ruminal fluid were slightly higher during casein infusion into the rumen than with no casein infusion. However, the concentrations of free AA did not increase as casein infusion increased. Concentration of ruminal ammonia increased as casein infusion into the rumen increased (Figure 1).

Urea concentration in blood plasma and NPN in milk were increased linearly as casein infusion into the abomasum and rumen (A100 and R100) increased (Figure 1). Urea concentration in blood plasma doubled from the lowest to the highest level of casein infusion. Although concentrations of urea in blood plasma increased during casein infusion into the abomasum (A100; Dhiman et al., 1993), no effect on ruminal ammonia concentration was detected, suggesting very little or no effect on urea recycling to the rumen even from the highest level of casein infusion. In earlier studies with sheep and cattle, no further increase in ruminal ammonia concentration occurred when plasma urea concentration increased above 4.3

Table 3. Milk yield, milk composition, and glucose concentration in blood plasma of cows during casein infusion into the abomasum and rumen[a]

Variable	Casein infused[b], kg/d					SEM
	0	.5	1.0	1.5	2.0	
Milk, kg/d	22.2	22.1	22.4	21.8	21.7	.8
3.5% FCM, kg/d[c]	20.7	20.7	20.9	20.4	20.4	.7
Milk fat, %	3.20	3.21	3.15	3.13	3.17	.06
Milk protein, %	3.24	3.28	3.30	3.29	3.27	.03
Fat yield, kg/d	.69	.69	.69	.68	.68	.02
Protein yield, kg/d	.71	.71	.73	.71	.71	.03
Plasma glucose, mg/dL	79.7	77.1	79.2	77.4	79.1	1.1

[a]Data were pooled across subperiods because there were no subperiod × treatment interactions ($P < .05$).
[b]In addition to the basal diet, cows were given casein infusions of 0, .5, 1.0, 1.5, and 2.0 kg/d. During subperiod 1, the full amount of casein was infused into the abomasum (A100); in subperiod 2, 10% of each casein level was infused into the abomasum (A10); and during subperiod 3, the full amount of casein was infused into the rumen (R100).
[c]3.5% FCM = .432 (kilograms of milk) + 16.2 (kilograms of fat).

Table 4. Rumen fermentation measurements of cows infused with casein into the rumen

Variable	Casein infused into the rumen, kg/d					SEM
	0	.5	1.0	1.5	2.0	
Ruminal pH	6.21	6.38	6.40	6.37	6.43	.1
Total VFA, μmol/mL	113.0	113.1	112.0	110.1	115.2	5.5
Individual VFA, mol/100 mol						
Acetate	64.8	62.9	64.1	60.6	61.3	1.1
Propionate	19.2	20.1	19.0	19.9	19.6	1.0
Isobutyrate	1.1[b]	1.4[b]	1.3[b]	2.2[a]	2.1[a]	.1
Butyrate	11.3	11.5	12.0	11.2	11.2	.3
Isovalerate	1.9[b]	2.3[b]	2.1[b]	3.6[a]	3.2[a]	.2
Valerate	1.7[b]	1.9[b]	1.6[b]	2.5[a]	2.5[a]	.2
Free AA, μmol/mL	.31	.46	.53	.47	.53	.08

[a,b]Means in the same row with different superscripts differ ($P < .01$).

m*M* (Thornton, 1970). Casein infusion into the abomasum at a rate equal to 10% of each infusion level (A10) increased the concentration of blood plasma urea and milk NPN at the highest level of infusion ($P < .05$). Urea concentration in blood plasma generally increases as the supply of protein in the rumen or intestine increases (Broderick et al., 1974). Part of this urea was secreted in milk, as indicated by an increased concentration of NPN in milk.

The concentrations of AA in blood plasma reflect the supply of these AA from intestinal absorption and endogenous synthesis as well as the demand for protein synthesis and degradative metabolism (Harper, 1968). Unlike other essential AA, the liver has less capacity to degrade BCAA (Harper et al., 1984), and concentrations of BCAA in blood plasma have therefore been used as an indicator of protein absorption from the intestine (Bergen et al., 1973). Concentration of essential AA and BCAA (sum of Ile, Leu, and Val) in blood plasma increased linearly ($P < .05$ and $P < .01$, respectively), as expected, as amounts of casein infusion into the abomasum increased (A100, Figure 2). However, when the same amount of casein was infused into the rumen (R100), essential AA and BCAA concentrations in plasma tended to decrease as casein infusion increased. This decrease was significant ($P < .01$) with infusion of the largest amount of casein into the rumen. It is not clear why a decrease occurred, unless it was caused by the slight reduction in feed intake at the two highest levels of casein infusion. Perhaps net microbial protein synthesis was reduced slightly by replacing some dietary carbohydrate with infused casein. Feed consumption was reduced ($P < .05$) at the highest level of casein infusion, and BCAA concentrations in blood plasma at the highest level of casein infusion could have been affected by this.

The absence of any increase in BCAA indicates that protein available at the small intestine probably was not increased because of an enhanced supply of RDP from the ruminally infused casein. Casein infusion into the abomasum at a rate equal to 10% of the rumen infusion resulted in an increase in BCAA concentration at the highest level of casein infusion ($P < .01$). We assumed that 10% of the casein infused into the rumen would escape degradation and reach the intestine, but apparently either less than 10% of the casein escaped ruminal degradation, or the small reduction in feed intake was responsible for the decrease in protein uptake from the intestine. Concentrations of nonessential AA in blood plasma during 0, .5, 1.0, 1.5, and 2.0 kg of casein infusion per day into the abomasum (A100) were 952, 925, 1,011, 975, and 1,090 μmol/L, respectively. Nonessential AA concentrations in blood plasma did not change during A10 or during casein infusion into the rumen (R100).

The results of this study indicate that the diet used, containing 14% CP and 9.3% RDP, supplied sufficient nitrogen precursors to support maximum microbial growth. Additional supply of RDP from the ruminally infused casein had no effect on protein flow to the small intestine. The RDP requirements, according to NRC (NRC, 1989), of a cow weighing 600 kg and producing 20 to 50 kg of 3.5% FCM/d range from 9.6 to 10.3% of dietary DM. This study and the study by Armentano et al. (1993) lend support to the view that current NRC recommendations allow for adequate RDP.

Implications

It is important to feed an adequate amount of ruminally degraded protein (RDP), but not an excess. The excess RDP is excreted in the urine and is potentially an environmental problem. Some have suggested that the National Research Council does not recommend sufficient amounts of RDP. This study supports the current National Research Council recommendation as being adequate in RDP for dairy cattle.

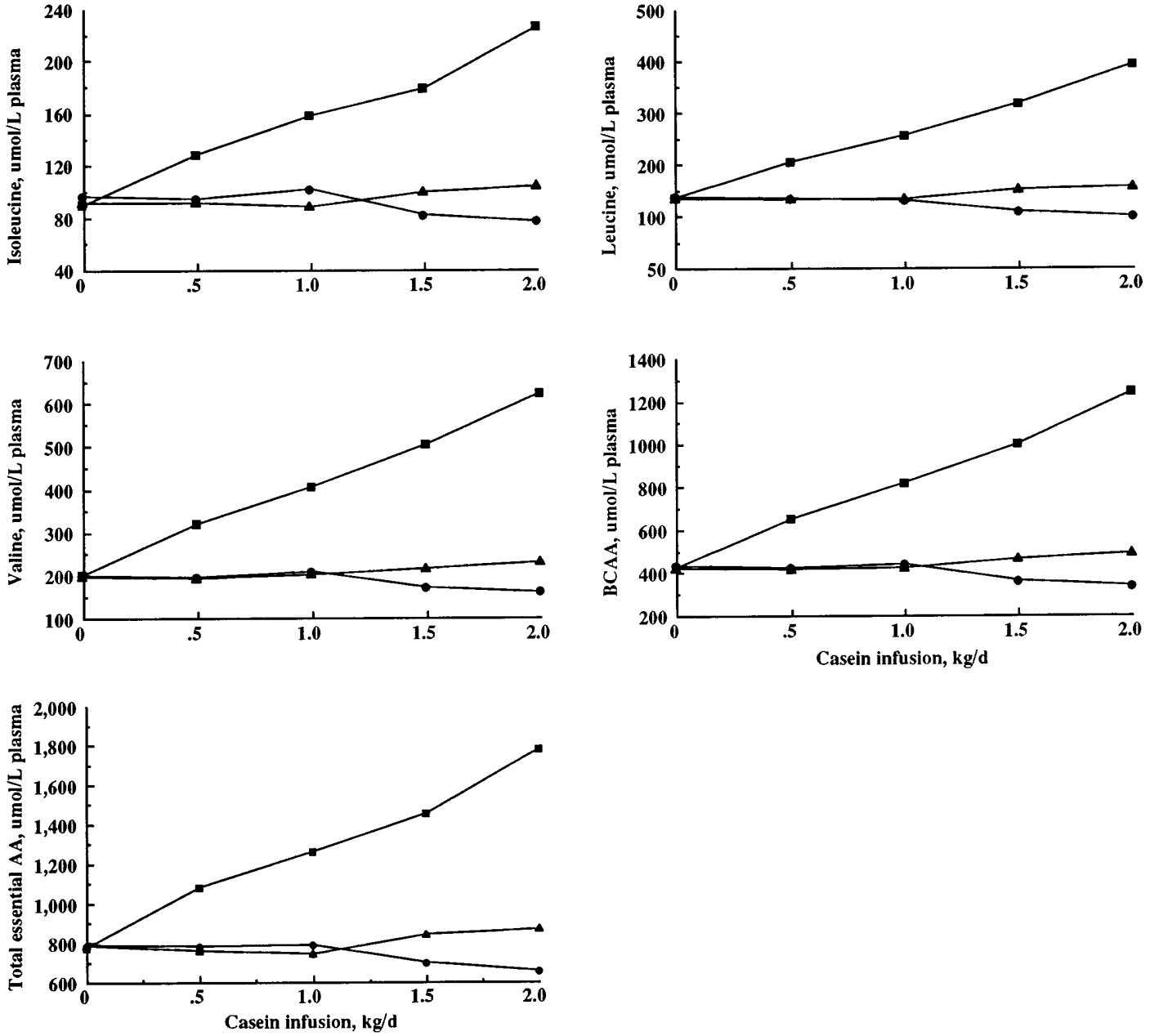

Figure 2. Effect of ruminally degraded protein on concentrations of individual, total branched-chain amino acids (BCAA; sum of Ile, Leu, and Val), and total essential AA (sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val) in blood plasma. In addition to the basal diet, cows were given casein infusions of 0, .5, 1.0, 1.5, and 2.0 kg/d. During subperiod 1, the full amount of casein was infused into the abomasum (A100, ■); in subperiod 2, 10% of each casein level was infused into the abomasum (A10, ▲); and during subperiod 3, the full amount of casein was infused into the rumen (R100, ●). The following SEM are for A100, A10, and R100: 8.2, 4.6, and 6.0 for Ile; 14.2, 6.4, and 6.2 for Leu; and 16.6, 8.0, and 10.4 for Val; 37.8, 18.6, and 21.8 for total BCAA; 56, 32, and 36 for total essential AA, respectively. The slopes of response lines (unit change in response due to a unit change in level of casein infusion) are given below.

		Subperiod		
Item	A100	A10	R100	SE of estimate
Isoleucine	16.17[a]	1.72[b]	–2.54[b]	1.03
Leucine	31.44[a]	3.24[b]	–4.97[b]	1.68
Valine	51.49[a]	4.49[b]	–4.96[b]	2.23
BCAA	99.11[a]	9.45[b]	–12.46[b]	4.76
Essential AA	119.2[a]	12.22[b]	–17.57[b]	7.14

[ab]Means with different superscript in the same row differ ($P < .05$).

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